

What is claimed is:

Sub B1
1. A method for simultaneously measuring both members A and B of a binding pair in a biological sample, said method comprising:

5 a) providing a solid phase reagent, said solid phase reagent comprising a particle coated with capture antibodies having specific binding affinities for said member A of said binding pair;

b) contacting said biological sample with said solid phase reagent under conditions in which said member A, if present, becomes bound to said particle, to form a first reacted particle;

10 c) contacting said first reacted particle with first antibodies having specific binding affinities for said member A, wherein said first antibodies are labeled with a first label, and with second antibodies having specific binding affinities for said member B of said binding pair, wherein said second antibodies are labeled with a second label, to form a second reacted particle, and

15 d) measuring said first and second labels on said second reacted particle using flow cytometry.

2. The method of claim 1, wherein (substantially all) said capture antibodies are oriented on said particle such that the antigen binding regions of said capture antibodies are available for binding said member A of said binding pair.

20 3. The method of claim 1, wherein said member A is an antigen and said member B is a host antibody.

4. The method of claim 3, wherein said antigen is a viral antigen.

5. The method of claim 4, wherein said viral antigen is a hepatitis C antigen.

6. The method of claim 4, wherein said viral antigen is a hepatitis B antigen.

5 7. The method of claim 4, wherein said viral antigen is a human immunodeficiency virus antigen.

8. The method of claim 1, wherein said antigen is an autoantigen.

9. The method of claim 8, wherein said autoantigen is glutamic acid decarboxylase.

10 10. The method of claim 1, wherein said member A is a ligand and said member B is a receptor.

11. The method of claim 10, wherein said ligand is a cytokine and said receptor is a cytokine receptor.

12. The method of claim 1, wherein said member A is an enzyme and said
15 member B is a substrate.

13. The method of claim 12, wherein said enzyme is caspase-3 and said substrate is poly(ADP-ribose) polymerase.

14. The method of claim 12, wherein said enzyme is caspase-1 and said substrate is proInterleukin-1.

15. The method of claim 1, wherein said first and second labels are fluorophores.

16. The method of claim 1, wherein said biological sample is selected from the group consisting of blood, plasma, serum, urine, cerebrospinal fluid, sputum,
5 tears, amniotic fluid, vitreous humor, saliva, and tissue culture supernatants.

17. The method of claim 1, wherein said capture antibodies are monoclonal.

18. The method of claim 1, wherein said first antibodies are monoclonal.

10 19. The method of claim 1, wherein said second antibodies are monoclonal.

20. A kit for simultaneously measuring both members A and B of a binding pair in a biological sample, said kit comprising:

15 a) a solid phase reagent, said solid phase reagent comprising a particle coated with capture antibodies having specific binding affinities for said member A of said binding pair, wherein (substantially all) said capture antibodies are oriented on said particle such that the antigen binding regions of said capture antibodies are available for binding said member A of said binding pair;

b) first antibodies having specific binding affinities for said member A of said binding pair, wherein said first antibodies are labeled with a first label; and

20 c) second antibodies having specific binding affinities for said member B of said binding pair, wherein said second antibodies are labeled with a second label.

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